Sunday, February 20
1:30 PM – 3:00 PM
Esplanade, Room 157
Mad City Labs Inc

Novel Single Molecule Methods for Tracking, Transport, and Protein Complex Analyses

Capturing the Early Stages of the Virus-Cell Interaction With Active-Feedback Single-Virus Tracking
Kevin Welsher
Here we introduce an active-feedback 3D microscopy technique capture the dynamics of rapidly diffusing single molecules in solution (3D Single-Molecule Active Real-time Tracking or 3D-SMART). This method “locks" target fluorophores in the focal volume of an optical microscope using real-time feedback to move the sample and compensate for molecular diffusion. 3D-SMART has been successfully applied to single proteins and nucleic acids at diffusive speeds up to 10 $\mu m^2/s$. We will further describe how this microscope, when combined with rapid volumetric imaging, can capture the early events in the interactions between single viral particles and live cells in three dimensions with millisecond or better temporal resolution.

Measuring Activity of Single Transporters in Single Vesicles Using TIRF Microscopy
Joseph Mindell
Secondary active transporters are essential contributors to ion and substrate fluxes across biological membranes. One limitation to our current knowledge is that transporter activity measurements generally reflect the average behavior of a multitude of proteins, while the individual behaviors of single transporters are unresolved. Here we report development of a novel, single-liposome assay using single-molecule techniques to measure the activity of individual transporters using ClC-ec1, a member of the CLC family of Cl-/H+ exchangers as a model system. Using this method, we observe transport catalyzed by single CLC-ec1 dimers and investigate the nature of stoichiometric coupling in this antiporter.

Rapid Extraction and Kinetic Analysis of Protein Complexes From Single Cells
Sena Sarıkaya & Dan Dickinson
Understanding assembly of molecular machines in developing cells requires a quantitative, biochemical, and *in vivo* approach. We developed an assay to interrogate the abundance, dynamics, and stability of native protein complexes extracted from single cells. We optically lysed *Caenorhabditis elegans* zygotes, captured *in vivo* single protein complexes on a coverslip using antibodies, and monitored the dynamics of these complexes over time using multi-color MicroMirror Total Internal Reflection Fluorescence microscopy. We developed open-source software to process thousands of kinetic measurements per cell. This assay provides an unprecedented level of resolution as compared to traditional *in vitro* biochemistry and can be applied to any protein pair that can be labeled and detected.

Speakers
Joseph Mindell, National Institute of Neurological Disorders and Stroke, NIH
Sena Sarıkaya, Department of Molecular Biosciences, The University of Texas at Austin
Kevin Welsher, Department of Chemistry, Duke University